FDDU
Procedures for Capillary Electrophoresis using the 3130xl or 3730

1 Scope

These procedures apply to the preparation of daughter plates of amplified samples followed by the separation by capillary electrophoresis (CE) with an AB 3130xl Series Genetic Analyzer or an AB 3730 Series DNA Analyzer in the Federal DNA Database Unit (FDDU).

2 Equipment/Materials/Reagents

Equipment/Materials

- General Laboratory Supplies (e.g., pipettes, tubes)
- Barcode printer with appropriately sized labels (2.0" x 0.5" or equivalent)
- Barcode Scanner, Hand-held (Symbol LS4000i, 4008i, LS4071 or equivalent)
- STaCS (Sample Tracking and Control System) Software (STaCS DNA Inc.), version 5.0 or higher
- Robotic Workstation (Tecan EVO 150/200)
  - Tecan EVOware Software, version 2.0 or higher (Tecan)
- 96-Well Sample (MicroAmp) Plates (Applied BioSystems or equivalent)
- 96-Well Plate Septa (Applied BioSystems or equivalent)
- Plate Sealer, microplate (Agilent Plate Loc or equivalent) with heat sealing plastic
- GeneAmp® PCR System 9700 Thermal Cycler (Applied BioSystems)
- 3130xl Genetic Analyzer (Applied BioSystems)
  - 3130xl Data Collection Software, version 3.0 or higher (Applied BioSystems)
  - 96-Well Plate Base and Retainer (Applied BioSystems or equivalent)
  - Capillary Array (3130xl/3100), 36 cm x 50 µm (Applied BioSystems)
- 3730 DNA Analyzer (Applied BioSystems)
  - 3730 Data Collection Software, version 3.0 or higher (Applied BioSystems)
  - 96-Well Plate Base and Retainer (Applied BioSystems or equivalent)
  - Capillary Array (3730), 36 cm (Applied BioSystems)

Reagents

- AmpF*l*STR® Identifiler® Allelic Ladder (Applied BioSystems)
- AmpF*l*STR® Identifiler® Direct Allelic Ladder (Applied BioSystems)
- Hi-Di™ Formamide (Applied BioSystems or equivalent)
- GeneScan 600 LIZ Size Standard (GS-600 [LIZ]) (Applied BioSystems)
- Bleach, 3% (household or equivalent)
- Liquinox™ Critical Cleaning Liquid Detergent (Alconox or equivalent)
- Water, reagent grade (VWR #48218-710 or equivalent)
- 10X Genetic Analyzer Buffer with EDTA (Applied BioSystems or equivalent)
- Performance Optimized Polymer, AB 3130xl POP-4® or AB 3730 POP-7® (Applied BioSystems)
- Matrix Standard Kit, Multi-Capillary DS-33 (Dye Set G5 and G5-RCT) (Applied BioSystems 4345833)

3 Standards and Controls

The following controls are included on each amplification plate using the listed kit and will be transferred for capillary electrophoresis. These controls will be interpreted according to the criteria in the applicable FDDU Procedure.

Identifiler (ID)	Identifiler Direct (IDD)
Negative	Negative (aka Combo)
Amp Blank	BIS
Positive (9947A)	
BIS	

4 Procedures

Refer to DNA Procedure Introduction (DNA QA 600) for applicable laboratory quality assurance and cleaning instructions.

When using a Robotic Workstation, ensure general instrument cleaning and maintenance is done prior to use, as needed. See the FDDU Procedure for FTA Preparation and STR Amplification (FDDU 305) Appendix A for additional guidance.

4.1 Electrophoresis Plate Preparation (EPP) Daughter Plate Creation

4.1.1 Create the daughter plate in STaCS.

4.1.2 Upon completion of the daughter plate creation, STaCS prints out plate barcodes with a daughter plate designation. Place each barcode accordingly, on the EPP MicroAmp plate and support base.

4.1.3 Scan the barcodes affixed to both the EPP MicroAmp plate and support base. STaCS verifies the scanned barcodes.

4.1.4 Repeat for each plate that is being processed at EPP.

4.2 GS-600 [LIZ] Formamide Preparation

4.2.1 Prepare the GS-600 [LIZ] formamide. The solution is prepared by combining Hi-Di formamide with GS-600 [LIZ] size standard in a 50:1 ratio. One extra plate should be included in the calculation for overage.

GS-600 [LIZ] Formamide (per 96-Well daughter plate)	
Hi-Di™ Formamide	2500 µl
GS-600 [LIZ] size standard	50 µl

4.2.2 Ensure the preparation has been recorded in the *Chemical Preparation* module of STaCS.

4.2.3 Store the GS-600 [LIZ] formamide in a refrigerator and use within the same day as it was prepared.

4.3 Electrophoresis Plate Preparation (EPP)

The EPP procedure will be performed based on the amplification kit and the sample type:

	Identifiler	**Identifiler Direct**
Blood	Manual or Automated	N/A
Buccal	Manual	Manual or Automated

4.3.1 Quick-spin the 96-Well Amplification plate(s) for approximately 30 seconds.

4.3.2 Within STaCS, select the daughter plate(s) to be processed and select the appropriate scenario.

4.3.2.1 Additionally, for automated processing only:

- Scan the instrument barcode on the Tecan EVO Robotic Workstation.
- Ensure the Robot Maintenance Checks have been performed.
- Indicate whether each check passed.

4.3.3 Scan the barcode on each of the 96-Well Amplification plate(s), the EPP Daughter plate(s) and each reagent required for the selected scenario.

4.3.4 Select "Process" and proceed with the EPP procedure.

4.3.4.1 For manual processing, remove the cover from the 96-Well Amplification plate.

4.3.4.2 Additionally, for automated processing only:

- STaCS launches the robotic software for the Tecan EVO Robotic Workstation
- If necessary, enter the appropriate user name and password at the robotic software log-in screen.

- Verify that the appropriate EPP script has been opened.
- Remove any plastic cover(s) on the 96-Well Amplification plate(s), if necessary.
- Ensure the selected 96-Well Amplification plate(s), the corresponding EPP Daughter plate(s) and the required reagents have been loaded on to the instrument.
    - Well A1 should be in the back right corner of the hotels.
- Ensure the instrument has been properly flushed and no air bubbles are visible in the tubing or syringes.
- Start the EPP script.
- Indicate the number of plates to be processed.

The following EPP procedure will be performed manually or by the Robotic Workstation:

4.3.5 Aliquot 24 µl of the GS-600 [LIZ] formamide solution into each well of the EPP Daughter plate(s).

4.3.6 Add 1 µl of PCR product from the 96-Well Amplification plate(s) to its corresponding sample well in the EPP Daughter plate(s) and 1 µl of the appropriate allelic ladder to the designated well(s) in the EPP Daughter plate(s).

4.3.7 The Tecan EVO Robotic Workstation will re-seal the 96-Well Amplification plate(s) with a plastic cover. For manually processed plate(s), heat seal the 96-Well Amplification plate(s) with a plastic cover.

4.3.8 Visually inspect the EPP Daughter Plate(s).

4.3.9 Indicate the result in STaCS as successful, failed or aborted. Comments and observations must be entered for plates with process failed results. If the plate(s) were processed on the Tecan EVO Robotic Workstation, indicate in STaCS whether the bleach process was performed.

4.3.10 Ensure the EPP Daughter plate(s) are covered with septa.

4.3.11 Vortex (approximately 2 seconds) and quick-spin (approximately 30 seconds) the EPP Daughter Plate(s).

4.3.12 Return the 96-Well Amplification plate(s) to refrigerated storage in the post-amplification laboratory (4°C ± 3°C) until data analysis has been completed, at which time the plate(s) can be discarded.

4.4 Electrophoresis Preparation Denature

4.4.1 Load the EPP Daughter Plate(s) into the Thermal Cycler(s).

4.4.2 Slide the lid of the thermal cycler(s) forward over the EPP Daughter Plate(s). Do not close the lid of the thermal cycler during the denaturing step.

4.4.3 Select the appropriate method on the thermal cycler and verify that the method displayed on the instrument screen matches the method outlined below:

HOLD 95°C 3 minutes
HOLD 4°C 3 minutes
HOLD 4°C Forever

4.4.4 Start the thermal cycler. Verify that the reaction volume is 25 µl and the ramp speed is 9600.

4.4.5 Ensure the *Thermal Cycler Bar Code* and *EPP Daughter Plate Bar Code* for each plate to be denatured has been scanned into STaCS.

4.4.6 Select "Save" and indicate the result in STaCS as successful, failed or aborted. Comments and observations must be entered for plates with process failed results.

4.5 Setting Up the Genetic Analyzer (Sequencer)

The type of AB Genetic Analyzer validated to perform capillary electrophoresis is based on the amplification kit and sample type as follows:

	Identifiler	**Identifiler Direct**
Blood	3130xl or 3730	N/A
Buccal	3130xl	3730

***NOTE**: If sequencer general maintenance is required, refer to Appendix A for guidance.*

4.5.1 Start the computer attached to an appropriate AB Genetic Analyzer and log onto the workstation.

4.5.2 Ensure that the oven and all instrument doors are shut. Press the power button on the front of the analyzer to start the instrument. Ensure that the green status light is on before proceeding.

4.5.3 Launch the Data Collection Software and Service Console application. The Data Collection software will open once a green box is indicated for the *Messaging Service*, *Data Service*, *Instrument Service*, and *Viewer*. Expand the navigation panel on the left.

4.5.4 Replenish the buffer, water and waste reservoirs, if necessary.

	3130xl Genetic Analyzer	**3730 Genetic Analyzer**
Frequency (*whichever comes first*)	One week **or** 6 full 96-Well plates (~36 injections)	48 hours **or** 16 full 96-Well plates (~32 injections)
Anode Buffer Reservoir (1X Genetic Analyzer Buffer with EDTA)	Fill to the red line (~16mL) Place on the pump block	Fill to the red line (~67 mL) Place on the pump block
Cathode Buffer Reservoir (1X Genetic Analyzer Buffer with EDTA)	Fill reservoir to the fill line (~16mL) Place in position 1 on the autosampler	Fill reservoir to the line (~80 mL) Place in the left most position
Water and Waste reservoirs (water)	Fill three reservoirs to the fill line (~16mL each) Place in positions 2 through 4 on the autosampler	Fill two reservoirs to the line (~80 mL each) Place in middle and right positions, respectively

- To prepare 1X Genetic Analyzer Buffer, dilute the appropriate 10X Genetic Analyzer Buffer with water and record in the *Chemical Preparation* module in STaCS.
- Ensure cathode buffer, water, and waste reservoirs are covered with septa.

NOTE: *To avoid electrical arcing, all surfaces of the reservoirs must be clean and dry.*

4.5.5 The oven may be turned on and the temperature set in advance to shorten the interval between run activation and execution. It takes approximately 30 minutes to reach the target temperature.

From the *Manual Control* menu in the navigation panel:

3130xl Genetic Analyzer	**3730 Genetic Analyzer**
o *Send defined command for* = Oven o *Command Name* = Turn on/off oven o *Value* = On o Execute by selecting "Send Command"	o *Send defined command for* = Oven o *Command Name* = Set oven state o *Value* = On o Execute by selecting "Send Command"
Set the oven to 60° C by selecting the following parameters: o *Send defined command for* = Oven o *Command Name* = Set oven temperature o *Value* = 60°C o Execute by selecting "Send Command"	Set the oven to 66° C by selecting the following parameters: o *Send defined command for* = Oven o *Command Name* = Set oven temperature o *Value* = 66°C o Execute by selecting "Send Command"
	To turn the buffer heater on, select the following parameters: o *Send defined command for* = Autosampler o *Command Name* = Turn On/Off buffer heater o *Value* = On o Execute by selecting "Send Command"

NOTE: *The oven and/or buffer reservoir status can be verified by selecting Instrument Status from the navigation panel.*

4.5.6 Ensure the chemicals/reagents/array required for the Genetic Analyzer have been defined and/or verified in STaCS using the *Sequencer Configuration* module.

4.5.7 If necessary, ensure any changes to chemicals/reagents/array performed on the Genetic Analyzer have been recorded in STaCS using the *Instrument Maintenance* module.

4.6 Post PCR and Sample Sheet Creation

In the *Post PCR* module, the type of Genetic Analyzer (*Sequencer*) to be used for capillary electrophoresis is selected, the reagents assigned to the sequencer are recorded and a sample sheet(s) generated.

4.6.1 Within STaCS, scan the *Sequencer Bar Code* of the selected instrument that will be utilized to perform capillary electrophoresis.

4.6.2 Scan the barcode on the Electrophoresis (EPP) daughter plate(s) to be processed.

4.6.3 Specify the *Destination Directory* for the sample sheet(s) in STaCS.

4.6.4 Create the sample sheets. STaCS creates a sample sheet file with the same name as its corresponding EPP daughter plate barcode.

NOTE: *If multiple injections of any FDDU samples and/or controls are required, the user may add the injections during the creation of the sample sheet or later in the Data Collection software of the sequencer. If extra injections are added during creation of the sample sheet, all samples and controls on the plate will have the additional injections included in the sample sheet.*

4.6.5 Select "Save" and indicate the result in STaCS as successful, failed or aborted. Comments and observations must be entered for plates with process failed results.

4.7 Initiating Capillary Electrophoresis on a 3130xl Genetic Analyzer

4.7.1 Import the STaCS generated sample sheets.

- Choose *Plate Manager* from the navigation panel.
- Select *Import* and browse to the STaCS generated sample sheet file(s).
- Select *OK* to import the Plate Record(s) into the 3130xl Data Collection Software.

4.7.2 In the Plate Manager window, verify the following:

- *Plate ID* and *Plate Name* = the corresponding STaCS daughter plate barcode
- *Type* = GeneMapper
- *Size* = 96

4.7.3 Review the Plate Record(s)

- In the *Plate Manager* window, highlight the plate and select *Edit* to open the *GeneMapper Plate Editor* spreadsheet
- Ensure the *Sample Name* column contains expected sample barcodes
- Ensure the *Sample Type* for Ladders and Controls and that the *Size Standard, Panel* and *Analysis Method* fields are complete
- Ensure the *Results Group* and *Instrument Protocol* fields are correct for the selected instrument
- Use the pull down menus to make any necessary changes to the spreadsheet

Note: *The parameters (e.g., Injection kV) defined in an Instrument Protocol/Panel may vary between AB 3130xl Genetic Analyzers. The Instrument Protocol/Panel(s) for each instrument are available for reference.*

4.7.4 If necessary, to add injections of any FDDU samples and/or controls not added during Post PCR and Sample Sheet Creation:

- Select "Add Sample Run" from the *Edit* menu to add additional *Results Group* and *Instrument Protocol* columns.
- Select the appropriate *Results Group* and *Instrument Protocol* parameters for the FDDU samples and/or controls that require additional injections.
- Select "OK" to save the Plate Record.

4.7.5 Ensure the AB 3130xl plate assemblies have been prepared with the EPP Daughter Plates and the plate assemblies are properly seated on the autosampler.

4.7.6 Select *Run Scheduler* from the navigation panel. In *Plate View*, the checkered box will change from gray to yellow when the plate assemblies are seated correctly. Link the EPP daughter plate(s) to their *Plate Record* (sample sheets).

- Select *Plate View* under the *Run Scheduler* on the navigation panel.
- Select the appropriate plate record for the EPP daughter plate in autosampler position *A*.
- Verify that the EPP daughter plate barcode in autosampler position *A* matches the selected *Plate Record*.
- Select the checkered area of the plate *A* position indicator.
- This activates the green *Start Run* icon located on the tool bar.
- Repeat, if necessary, to link the EPP daughter plate in position B.

4.7.7 Ensure that there are no bubbles in the tubing, array port or pump blocks. The Data Collection wizard for bubble removal may be run, if necessary. The capillary array may be filled with fresh polymer prior to starting the run.

- Choose *Manual Control* from the navigation panel
- *Send defined command for* = Polymer Delivery Pump
- *Command Name* = Fill 36 cm Capillary Array
- Execute by selecting "Send Command"

4.7.8 Ensure the doors on the AB 3130xl are closed and start the run.

- The Processing Plates dialog box opens and prompts the user that "You are about to start processing plates…"
- Select "OK"

Note: The electrophoresis run can be monitored by selecting *Instrument Status* from the navigation panel.

4.8 Initiating Capillary Electrophoresis on a 3730 DNA Analyzer

4.8.1 Import the STaCS generated sample sheets.

- Choose *Plate Manager* from the navigation panel.
- Select *Import* and browse to the STaCS generated sample sheet file(s).
- Select *Open* to import the Plate Record(s) into the 3730 Data Collection Software

4.8.2 In the Plate Manager window, verify the following:

- *Plate ID & Plate Name* = the corresponding STaCS daughter plate barcode
- *Type* = GeneMapper
- *Size*=96

4.8.3 Review the plate record for a minimum of one plate in each run:

- In the *Plate Manager* window, highlight the plate and select *Edit* to open the *GeneMapper Plate Editor* spreadsheet.
- Verify the *Sample Name* column contains expected sample barcodes.
- Verify the *Sample Type* for Ladders and Controls and that the *Size Standard, Panel* and *Analysis Method* fields are complete.
- Verify the *Results Group* and *Instrument Protocol* fields are correct for the selected instrument.
- Use the pull down menus to make any necessary changes to the spreadsheet.

NOTE: The parameters (e.g., Injection kV) defined in an *Instrument Protocol/Panel* may vary between AB 3730 DNA Analyzers. The *Instrument Protocol/Panel(s)* for each instrument are available for reference.

4.8.4 If necessary, to add injections of any FDDU samples and/or controls not added during Post PCR and Sample Sheet Creation:

- Select "Add Sample Run" from the *Edit* menu.
- Additional *Results Group* and *Instrument Protocol* columns are added to the *Genemapper Plate Editor* spreadsheet.
- Select the appropriate Results Group and Instrument Protocol parameters for only the FDDU samples and/or controls that require additional injections.
- Select "OK" to save the Plate Record.

4.8.5 Ensure the AB 3730 plate assemblies have been prepared with the EPP Daughter Plates and place the assemblies into the In Stacker tower on the plate stacker, which can hold up to 16 plates.

4.8.6 Verify that the EPP daughter plate barcode for each plate is clearly visible when the door to the In Stacker tower is open. This may require adjusting the barcode(s) to ensure they can be read by the internal barcode scanner.

4.8.7 Ensure the Out Stacker tower has sufficient space to process all plates to be run, close both tower doors and close the stacker drawer. This will home the autosampler causing the array to rest in the buffer reservoir.

4.8.8 Ensure the instrument Run Mode is on Auto:

- Select *Run Scheduler* from the navigation panel.
- From the *Instrument* menu select *3730_# Run Mode.*
- Verify that *Auto* is selected.
- When *Auto* mode is selected and the EPP daughter plate assemblies are loaded into the plate stacker; the *Plate Id*, *Plate Name* and *Plate Type* columns in the Input Stack portion of the *Run Scheduler* window will read *Unknown.*

4.8.9 Ensure the Output Stack portion of the *Run Scheduler* window has sufficient space to process all plates to be run.

4.8.10 Ensure there are no bubbles in the tubing, array port or pump blocks. The Data Collection wizard for bubble removal may be run, if necessary.

4.8.11 Ensure the correct Dye Set and Spectral are set for the run by checking the *Dye Set* and *Active Calibration* in the *Spectral Viewer* window.

4.8.12 Start the Run.

- The Processing Plates dialog box opens and prompts the user that "You are about to start processing plates…"
- Select "OK".

NOTE: *The electrophoresis run can be monitored by selecting Instrument Status from the navigation panel.*

5 Sampling

Not applicable.

6 Calculations

Not applicable.

7 Measurement Uncertainty

Not applicable.

8 Limitations

The appropriate processing methods are selected for a plate based on the sample type added to the plate and the amplification kit to be used. Based on internal studies, only the combinations of processes listed below are approved for use.

Sample Type	Amp Kit	EPP	Genetic Analyzer
Blood	Identifiler	Manual or Automated using a Tecan EVO Robotic Workstation	AB 3130xl or AB 3730
Buccal	Identifiler	Manual only	AB 3130xl
	Identifiler Direct	Manual or Automated using a Tecan EVO Robotic Workstation	AB 3730

9 Safety

9.1 Refer to the "Hazardous Waste Disposal" section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures as well as the biohazardous wastes generated.

9.2 Procedural Specific Chemical Hazard:

- Formamide is a teratogen. Avoid inhalation, skin contact, or ingestion. Use nitrile gloves when handling. Dispose of unused portions in appropriate hazardous waste containers. Pregnant women must not handle formamide.
- Performance Optimized Polymer 4 is caustic. Avoid inhalation, skin contact, or ingestion. Use gloves when handling. Dispose of unused portions in appropriate hazardous waste containers.
- Performance Optimized Polymer 7 is caustic. Avoid inhalation, skin contact, or ingestion. Use gloves when handling. Dispose of unused portions in appropriate hazardous waste containers.

10 References

FBI Laboratory Quality Assurance Manual.

FBI Laboratory Safety Manual.

DNA Procedures Manual.

Federal Bureau of Investigation Quality Assurance Standards for DNA Databasing Laboratories, current version.

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Wang et al. Development and Validation of the AmpFlSTR® Identifiler® Direct PCR Amplification Kit: Multiplex Assay for the Direct Amplification of Single-Source Samples, *Journal of Forensic Science* (2011) doi: 10.1111/j.1556-4029.2011.01757.x.

Rev #	Issue Date	History
7	06/26/14	Added clarification that control samples typed through capillary electrophoresis are included as applicable in section 4. Updated section number to reference in section 7.1.7 to reflect changes in numbering to this revision. Deleted need for instrument from sections 7.1.8 and 7.4.3.6. Deleted section 7.2.6 and associated footnotes and renumbered remainder of section. Deleted footnote 5. Deleted Appendix A and all associated references throughout document.
8	03/11/16	Revised entirety of document for simplification, clarity, and to remove computer interface requirements. Combined all FDDU post amplification laboratory procedures (306-7, 307-7, and 314-5). Relocated QA/QC guidance and procedures to Appendix A.

Approval

Redacted - Signatures on File

Appendix A: CE Maintenance and Performance Verification Procedures

General maintenance and performance verification (PV) procedures are performed in accordance with the LOM practice and DNA procedure for equipment calibration and maintenance. General maintenance is generally performed at the intervals listed below. Performance verification of the genetic analyzer instruments will be conducted at the minimum frequency described in the DNA procedure for equipment calibration and maintenance.

General Maintenance	Recommended Interval	
	3130 xl	3730
Replace Polymer	Weekly	Weekly
Replace 1x Buffer/Water/ Waste	Weekly (or as required in procedure)	Weekly (or as required in procedure)
Water Wash	Weekly	Weekly
Flush Water Trap	Weekly	Weekly
Database Cleanup	Monthly	Weekly
Data Backup	Monthly	Weekly
Disk Defragmentation	Monthly	Monthly
Install New Array	As needed	As needed
Spatial Calibration	With array change or as needed	Weekly
Spectral Calibration	As needed	Weekly
Sensitivity Evaluation	After optical adjustment	Quarterly or after optical adjustment

I. General Maintenance of the Applied Biosystems 3130*xl*

Weekly

1. Preparing new bottle of POP-4
    a. Loosen the POP-4 bottle cap and allow it to sit on the bench top for approximately 15 minutes to degas.
2. Flush the polymer delivery pump (PDP)
    a. Run the "water wash wizard" and use reagent grade water to flush the PDP. Note: For a warm water wash, heat water to < 60 ^{o}C.
    b. Follow wizard prompts.
3. Flush the PDP water trap
    a. Use a 20 mL Luer lock syringe filled with reagent grade water.
    b. Attach the syringe to the forward facing Luer fitting at the top of the pump block, open the Luer approximately one-half turn counter clockwise.
    c. Open the exit fitting at the top left side of the pump block approximately one-half turn counter clockwise.
    d. Flush the water trap with approximately 5 mL of water
    e. Close both fittings by turning them clock wise until finger-tight, do not over tighten.

Monthly

1. Maintenance of the storage databases used by the Data Collection software.
    a. Open the appropriate results group folder and create a new backup folder using the naming convention, CE #XX_Backup_MMDDYY.
    b. Move all plate folders into the newly created backup folder and then copy it to the appropriate CE Backup folder on the network. (Example: \\FS1\CE Backup)
2. Delete records from the database
    a. From the navigation pane, select "Database Manager" and "Cleanup Processed Plates".
    b. Allow the software the appropriate amount of time to delete the associated records and close the dialog box once complete.
3. Defragment the data storage hard drive using the disk defragmenter in Windows system tools to defragment the (E:) drive.

II. General Maintenance of the Applied Biosystems 3730

Weekly

1. Preparing new bottle of POP-7
    a. Loosen the POP-7 bottle cap and allow it to sit on the bench top for approximately 15 minutes to degas.
2. Flush the polymer delivery pump (PDP)
    a. Run the "water wash wizard" and use reagent grade water to flush the PDP. Note: For a warm water wash, heat water to < 60 ^{o}C.
    b. Follow wizard prompts.
3. Flush the PDP water trap
    a. Use a 20 mL Luer lock syringe filled with reagent grade water.
    b. Attach the syringe to the forward facing Luer fitting at the top of the pump block, open the Luer approximately one-half turn counter clockwise.
    c. Open the exit fitting at the top left side of the pump block approximately one-half turn counter clockwise.
    d. Flush the water trap with approximately 5 mL of water
    e. Close both fittings by turning them clock wise until finger-tight, do not over tighten.
4. Perform a spatial calibration upon completion of the "water wash wizard".
    a. Refer to spatial calibration procedure at the end of the appendix for instructions and pass/fail criteria.
5. Perform a spectral calibration following successful spatial calibration.
    a. Refer to spectral calibration procedure at the end of the appendix for instructions and pass/fail criteria.
6. Maintenance of the storage databases used by the Data Collection software.
    a. Open the appropriate results group folder and create a new backup folder using the naming convention, CE#XX_Backup_MMDDYY.
    b. Move all plate folders into the newly created backup folder and then copy it to the appropriate CE Backup folder on the network. (Example: \\FS1\CE Backup)
7. Delete records from the database
    a. From the navigation pane, select "Database Manager" and "Cleanup Processed Plates".

b. Allow the software the appropriate amount of time to delete the associated records and close the dialog box once complete.

Monthly

1. Defragment the data storage hard drive using the disk defragmenter in Windows system tools to defragment the (E:) drive.

III. Array Change and Spatial Calibration

The capillary array will be changed as needed. The determination to change the array will be based upon a review of the quality of the data generated by the instrument. Generally, the array on the 3130xl should be changed after 150 injections.

1. From the toolbar select the "Install Array Wizard".
2. Install the array as instructed by the wizard.
    a. Ensure the proper type (16 or 48) and length (36 cm) is entered in the array information fields.
3. In the final step of the wizard you can choose to fill the array with polymer or click "finish" if the array will be filled during the spatial calibration.

A spatial calibration must be performed whenever a new array is installed, every time the detection cell window is opened, or each week for a 3730.

1. Select "Spatial Run Scheduler" in the navigation pane.
2. Select "SpatialFill_1"
3. Click the "Start" button to initiate the spatial calibration.
    Note: "SpatialNoFill_1" can be selected if there is no need to fill the array with fresh polymer.
4. Select "Accept" to accept the spatial calibration if the following criteria are met:
    a. Peaks of the spatial calibration are approximately the same height.
    b. An orange cross appears at the top (apex) of each peak in the profile.
    c. No irregular peaks are contained in the profile
    d. RFU values for the peaks are greater than 2,000 for a 3130 array and 1,000 for a 3730 array
    e. The values for the Left Spacing and Right Spacing columns for a 3130 array are 13-16 pixels and 9-11 pixels for a 3730 array. (A spatial calibration can be accepted if one or more of the spacing values lie outside of this range but it is preferable to have all the values within this specification.)

IV. Spectral Calibration

A spectral calibration is generally run each quarter for a 3130 and weekly for a 3730. While not necessary, a spectral calibration is recommended after changing the capillary array. A spectral plate may be injected several times within a 24 hour period, a fresh spectral plate should be used for each instrument being calibrated.

3130*xl*	**3730**
1. Combine 195 uL of formamide with 5 uL of DS-33 Matrix Standard 2. Dispense 10 uL of solution into wells A1-H1 and wells A2-H2. 3. Spin down and denature plate on thermal cycler then place on instrument. 4. Click "Plate Manager" in the navigation pane.* 5. Select "New" and the "New Plate Dialog" dialog box will open, fill out fields as follows a. Name: Use the naming convention CE#XX_Spectral_MMDDYY. b. Select "Spectral Calibration" from the "Application" drop down menu. c. Complete the remaining fields and select "OK". This will open the "Spectral Calibration Plate Editor" window. 6. Create sample sheet. a. Fill out the "Sample Name" fields to mirror the plate layout. b. Select "Spectral_G5" from the drop down in the "Instrument Protocol 1" field. c. Press "OK" to save plate sample sheet. 7. Select "Run Scheduler" from the navigation pane a. Search the plate name or select find all and click on the plate to be run in order to highlight it within the list. b. Click "Link" to associate the sample sheet to the plates location on the instrument. 8. Click the green arrow to start processing the spectral plate.	1. Combine 987 uL of formamide with 13 uL of DS-33 Matrix Standard 2. Dispense 10 uL of solution into wells A1-H1, A3-H3, A5-H5, A7-H7, A9-H9, and A11-H11 3. Spin down and denature plate on thermal cycler then place on instrument. 4. Click "Plate Manager" in the navigation pane.* 5. Select "New" and the "New Plate Dialog" dialog box will open, fill out fields as follows a. Name: Use the naming convention CE#XX_Spectral_MMDDYY. i. enter the same information in the "ID (Barcode)" field if running in manual mode, otherwise enter the plates barcode ID in this field. b. Select "Spectral Calibration" from the "Application" drop down menu. c. Select "Septa" from the "Plate Sealing" drop down menu d. Complete the remaining fields and select "OK". This will open the "Spectral Calibration Plate Editor" window. 6. Create sample sheet. a. Fill out the "Sample Name" fields to mirror the plate layout. b. Select "Spectral_G5-RCT" from the drop down in the "Instrument Protocol 1" field. c. Press "OK" to save plate sample sheet. 7. Select "Run Scheduler" from the navigation pane a. Search the plate name or select find all and click on the plate to be run in order to highlight it within the list. b. Click "Add" to add the plate to the input stack. 8. Click the green arrow to start processing the spectral plate.

* A previously created spectral plate can be duplicated by highlighting a plate in the Plate Manager window and clicking "Duplicate". This method will only require a new plate name be entered while retaining all the previously entered information.

Pass Criteria: The data collection software indicates the pass/fail status of each capillary. The spectral calibration is acceptable if the following criteria are met, and there is proper separation between the color channels.

Instrument	Number of failing wells	Minimum Peak height
3130*xl*	≤ 3caps, no more than 2 in a row	2,000 RFU
3730	≤ 5 caps, no more than 2 in a row	500 RFU

The Data Collection software automatically applies a passing spectral and no further action is required by the user.

V. Performance Verification and Sensitivity Evaluation

Performance verification of the genetic analyzer instruments should be conducted at the minimum frequency described in the DNA procedure for equipment calibration and maintenance. A Sensitivity Evaluation may be run in lieu of a Performance Verification and must be run after any optical adjustment is made to the instrument.

3130*xl*	**3730**
1. Amplify 9947A (Diluted/Promega) with the Identifiler amplification kit for 24 cycles. 2. Prepare a stock solution in a 24:1 ratio of GS600LIZ/Formamide to amplicon. 3. Add 25 uL of stock solution to wells A1-G1 and A2-G2, 4. Add 1 uL of Identifiler ladder and 24 uL of GS600LIZ/Formamide solution to wells H1 and H2. 5. Inject the samples three times at the instruments current injection setting.	1. Amplify 9947A (Diluted/Promega) with the Identifiler amplification kit for 24 cycles. 2. Prepare a stock solution in a 24:1 ratio of GS600LIZ/Formamide to amplicon. 3. Add 25 uL of stock solution to wells A1-G1, A3-H3, A5-G5, A7-H7, A9-H9, A11-G11. 4. Add 1 uL of Identifiler ladder and 24 uL of GS600LIZ/Formamide solution to wells H1, H5, and H11. 5. Inject the samples two times at the instruments current injection setting
Additionally for Sensitivity Evaluation:	
6. Inject the samples three times, at various voltage settings above and/or below the previously determined injection voltage.	6. Inject the samples two times, at various voltage settings above and/or below the previously determined injection voltage.

Pass Criteria: A Genetic Analyzer will be deemed suitable for FDDU analysis if:
1. Average peak heights of all alleles in the 9947A positive control are greater than 150 RFU and
2. Correct typing results obtained for 9947.

Injection voltage may be adjusted based on the evaluation of the data generated at the various injection voltage settings.